

(19)



Europäisches Patentamt

European Patent Office

Office européen des brevets

(11)

Publication number:

**0 090 417**  
**A2**

(12)

## EUROPEAN PATENT APPLICATION

(21)

Application number: **83103128.1**

(51)

Int. Cl.<sup>3</sup>: **C 12 P 19/40**  
**// C12N9/10**

(22)

Date of filing: **25.03.83**

(30)

Priority: **01.04.82 JP 55078/82**  
**04.11.82 JP 194394/82**

(71)

Applicant: **Yamasa Shoyu Kabushiki Kaisha, 10-1,  
Araoi-Cho 2-Chome, Choshi-Shi Chiba-Ken (JP)**

(43)

Date of publication of application: **05.10.83**  
**Bulletin 83/40**

(72)

Inventor: **Fujishima, Tetsuro, 8539-8, Kobatake Shin  
Machi, Choshi-Shi Chiba-Ken (JP)**  
Inventor: **Sakata, Shinji, 2-2, Sakae-Cho 2-Chome,  
Choshi-Shi Chiba-Ken (JP)**

(84)

Designated Contracting States: **CH DE FR GB LI**

(74)

Representative: **Dr. Elisabeth Jung Dr. Jürgen  
Schirdewahn Dipl.-Ing. Claus Gernhardt, P.O.  
Box 40 14 68 Clemensstrasse 30,  
D-8000 München 40 (DE)**

(54)

**Process for producing 3'-deoxyguanosine.**

(57)

Glycosylation or transglycosylation of a specified guanine derivative, namely 9-substituted or non-substituted guanine of formula [I] with a 3-deoxyribose donor such as 3'-deoxyadenosine in the presence of a nucleoside phosphorylase source such as of microorganism origin is disclosed.

**EP 0 090 417 A2**

## PROCESS FOR PRODUCING 3'-DEOXYGUANOSINE

BACKGROUND OF THE INVENTION

5 This invention relates to an enzymatic process  
for producing 3'-deoxyguanosine.

3'-Deoxyguanosine is a compound presently  
attracting attention which not only exhibits radiation  
sensitizing action in therapy of cancers but also has  
an action to enhance the effect of various anticancer  
10 agents when employed in combination therewith (see  
Japanese Laid-open Publication No. 35516/1982).

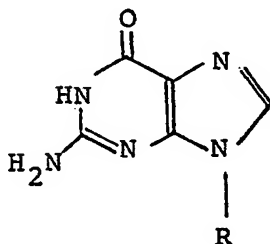
In the prior art, as the method for preparation of  
3'-deoxyguanosine, is known the method in which a  
chloromercuri of 2-acetamidohypoxanthine is condensed  
15 with 2,5-di-O-benzoyl-3-deoxy-D-ribofuranosyl bromide  
[The Journal of Organic Chemistry, 30, 2851 (1965)].  
However, this method is believed to involve various  
drawbacks in commercial production such as, for example,  
difficult availability of the starting material 3-  
20 deoxyribose, formation of isomers during the condensation  
reaction, use of a harmful mercuric salt, and others.

SUMMARY OF THE INVENTION

The present inventors have made various investi-  
gations in order to overcome these drawbacks of the  
25 prior art and consequently found that 3'-deoxyguanosine  
can be formed by causing a guanine derivative to react  
with a 3'-deoxyribose donor in the presence of a

nucleoside phosphorylase source. The present invention has been accomplished based on such a finding.

The present invention provides a process for producing 3'-deoxyguanosine, which comprises causing  
 5 a guanine derivative represented by the formula [I]:



[I]

10

wherein R designates a hydrogen atom or a ribose-1-yl group, a 2-deoxyribose-1-yl group or a monophosphate, a diphosphate or a triphosphate thereof, to react with  
 15 a 3-deoxyribose donor in the presence of a nucleoside phosphorylase source to obtain 3'-deoxyguanosine.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention is to be described in detail  
 20 below.

#### Guanine derivative

As the starting guanine derivatives, there may be employed one or more of the compounds included within the aforementioned definition. More specifically,  
 25 illustrative of these compounds are guanine (Gua), guanosine (Guo) or 2'-deoxyguanosine (2'-dGuo) or monophosphates, diphosphates or triphosphates thereof. The above nucleotide may have a phosphoryl group at any

position of hydroxyl groups in the sugar residue.

Typical examples of these nucleotides may include

guanosine-5'-monophosphate (GMP), guanosine-3'-mono-  
phosphate, guanosine-2'-monophosphate, guanosine-5'-

5 diphosphate (GDP), guanosine-5'-triphosphate (GTP),

2'-deoxyguanosine-5'-monophosphate (2'-dGMP), 2'-

deoxyguanosine-3'-monophosphate, 2'-deoxyguanosine-5'-

diphosphate (2'-dGDP), 2'-deoxyguanosine-5'-tri-

phosphate (2'-dGTP) and the like, which may be either

10 in free acid form or in an appropriate salt form such  
as sodium salt.

#### 3-Deoxyribose donor

As the other starting material, a 3-deoxyribose  
donor, there may be employed any compounds which are

15 capable of enzymatically introducing 3-deoxyribose

moiety to the 9-position of the guanine moiety of the

compound of formula [I] through direct glycosylation

or transglycosylation. Typical examples include one

or more of 3'-deoxyadenosine (3'-dAdo; cordycepin),

20 3'-deoxyinosine (3'-dIno), or monophosphates, diphos-

phates or triphosphates thereof, or 3-deoxyribose-1-

monophosphate. The above phosphates may have phosphoryl

group at any position of hydroxyl group in 3-deoxyribose,

and may also be either in free acid form or in a salt

25 form. Illustrative of these nucleotides are 3'-

deoxyadenosine-5'-monophosphate (3'-dAMP), 3'-

deoxyinosine-5'-monophosphate (3'-dIMP) or 3-deoxyribose-

1-phosphate.

Nucleoside phosphorylase source

The nucleoside phosphorylase which is in the nucleoside phosphorylase source in the reaction of the present invention refers comprehensively to a single enzyme or a plurality of enzymes capable of providing 3'-deoxyguanosine by causing the guanine derivative to react with the 3-deoxyribose donor in the presence of a phosphoric acid ion donor. Accordingly, in the present invention, the term "nucleoside phosphorylase source" includes the enzymes of the phosphorylase type such as purine nucleoside phosphorylase, pyrimidine nucleoside phosphorylase, etc., which can be used in combination with enzymes such as nucleoside-N-glycosyl transferase, nucleosidase, nucleotidase, phosphatase and others, which may possibly participate in the reaction of the present invention. The nucleoside phosphorylase source refers comprehensively to a material containing such enzymes in any desired form, which is not limited in its origin or source. That is, any enzyme material may be applicable so long as it can accomplish the object of the present invention, irrespective of whether it may be derived from microorganisms or from animals, or whether it may be prepared in any form. In particular, a nucleoside phosphorylase source of a microorganism origin, namely a nucleoside phosphorylase source in the form of a culture, a mass of intact cells or a modification of

cells of a microorganism, is preferred.

Preferred nucleoside phosphorylase sources of microorganism origin are those based on microorganisms which belong to genera Pseudomonas (hereinafter referred to as Ps.), Brevibacterium (hereinafter referred to as Br.), Achromobacter (hereinafter referred to as Ach.), Xanthomonas (hereinafter referred to as X.), Micrococcus (hereinafter referred to as M.), Staphylococcus (hereinafter referred to as St.) and Sarcina (hereinafter referred to as Sa.).

Typical strains of such microorganisms are:

Ps. desmolytica J-4-2: FERM P-6307, ATCC 39310;  
Br. acetylicum AT-6-7: FERM P-6305, ATCC 39311;  
Ach. eurydice BE-3-3: FERM P-6304, ATCC 39312;  
X. campestris: IAM 1671, ATCC 7381 (FERM P-6782);  
M. luteus: ATCC 4698, IAM 1056;  
St. aureus: IAM 1011, ATCC 6538P;  
St. aureus: IFP 3060;  
St. epidermidis: IFO 3762, ATCC 155; and  
Sa. marginata: FERM P-6539.

Typical strains among these are the first three strains, namely J-4-2 strain isolated from the soil in Nishiashikajima-Cho, Choshi-Shi, Chiba-Ken, Japan; the AT-6-7 strain isolated from the sand in the baseball ground of Koshien, Nishinomiya-Shi, Hyogo-Ken, Japan; and the BE-3-3 strain isolated from the soil in Araoi-Cho, Choshi-Shi, Chiba-Ken, Japan. The bacteriological

properties of these strains are set forth below.

1. J-4-2 strain

A. Morphology:

- (1) Form and size of cells: rod-shaped,  
5                      0.7 - 0.8 x 2.0 - 3.0  $\mu$ m;
- (2) Motility, occurrence of flagella: motile  
with polar flagella;
- (3) Formation of spores : none
- (4) Gram staining : negative

10        B. Growth on various culture media:

(1) Bouillon-agar plate culture (28°C, 48 hours)

- ① Form of colony : circular
- ② Raising of colony surface : raised or  
umbonate
- 15        ③ Size : 2 - 4 mm
- ④ Color tone : gray to buff when wet

(2) Bouillon-agar slant culture (28°C, 48 hours)

- ① Growth : good
- ② Form of growth : filliform and slightly  
20        spreading
- ③ Formation of pigment : not clear

(3) Bouillon liquid culture (28°C, 72 hours)

- Growth : no membrane formation, turbid through-  
out the entire liquid, sediment  
25        slightly formed.

(4) Bouillon-gelatin stab culture (20°C, 6 days) :  
not liquefied

0090417

(5) Litmus-milk culture medium (28°C, 4 days):  
not changed.

C. Physiological properties:

(1) Reduction of a nitrate (28°C, 5 days) :  
reductive

(2) Formation of hydrogen sulfide (28°C, 5 days):  
formed

(3) Hydrolysis of starch : not hydrolyzed

(4) Catalase : positive

10 (5) Indole formation : negative

(6) Ammonia formation from peptone and arginine :  
negative

(7) Methyl red test : negative

(8) V-P test : positive

15 (9) Attitude to oxygen : aerobic

(10) O-F test (by the Hugh Leifson method) : O type  
(Oxidation)

(11) Acid formation from sugars

positive : glucose, mannose, fructose, maltose,  
20                      saccharose, trehalose and mannitol;

negative : arabinose, xylose, galactose, lactose,  
sorbitol, inositol and glycerine

(12) Growth pH range : pH 6.0 - 9.0

(13) Optimum growth temperature : 25 - 35°C

25      2.    AT-6-7 strain

A. Morphology:

(1) Form and size of cells: short rod-shaped,



0.8 - 1.0 x 1.0 - 1.2  $\mu$ m;

(2) Formation of spores : none

(3) Gram staining : positive

B. Growth on various culture media:

5 (1) Bouillon-agar plate culture (28°C, 48 hours)

① Form of colony : circular

② Raising of colony surface : flat, smooth

③ Size : 2 - 4 mm

④ Color tone : yellow to peach-yellow

10 (2) Bouillon-agar slant culture (28°C, 48 hours)

① Growth : good

② Form of growth : echinulate

(3) Bouillon liquid culture (28°C, 48 hours)

Growth : formation of ring on the surface,  
sediment slightly formed.

15

(4) Bouillon-gelatin stab culture (20°C, 6 days):

liquefied in stratiform

(5) Litmus-milk culture medium (28°C, 4 days):

slightly coagulated, peptonization also observed

20

C. Physiological properties:

(1) Reduction of a nitrate (28°C, 5 days) :  
no reductivity

(2) Formation of hydrogen sulfide (28°C, 5 days) :  
not formed

25

(3) Hydrolysis of starch: hydrolyzed

(4) Catalase : positive

(5) Indole formation : not formed

- (6) Ammonia formation from peptone and arginine : negative
- (7) Methyl red test : negative
- (8) V-P test : positive
- 5 (9) Attitude to oxygen : aerobic
- (10) O-F test (by the Hugh Leifson method) : F type (Fermentation)
- (11) Acid formation from sugars  
positive : glucose, mannose, fructose,  
10 maltose, saccharose and trehalose;  
negative : arabinose, xylose, galactose, lactose,  
sorbitol, inositol and glycerine
- (12) Growth pH range : pH 6.0 - 9.0
- (13) Optimum growth temperature : 25 - 37°C
- 15 3. BE-3-3 strain
- A. Morphology:
- (1) Form and size of cells : rod-shaped,  
0.8 - 0.9 x 1.4 - 1.8  $\mu$ m;
- (2) Formation of spores : none
- 20 (3) Gram staining : negative
- B. Growth on various culture media:
- (1) Bouillon-agar plate culture (28°C, 48 hours)
- ① Form of colony : rhizoid and lacerate
- ② Raising of colony surface : flat and smooth
- 25 ③ Size : 5 - 9 mm
- ④ Color tone : pale grayish brown to  
bluish gray

0090417

- (2) Bouillon-agar slant culture (28°C, 48 hours)

① Growth : good

(2) Form of growth : filiform

- (3) Bouillon liquid culture (28°C, 48 hours)

Growth : turbid throughout the entire  
liquid, sediment formed.

- (4) Bouillon-gelatin stab culture (20°C, 6 days) :  
not liquefied

- (5) Litmus-milk culture medium (28°C, 4 days) :  
10 substantially not changed

C. Physiological properties:

- (1) Reduction of nitrate (28°C, 5 days) :  
no reductivity

- (2) Formation of hydrogen sulfide (28°C, 5 days) :  
15                   formed

- (3) Hydrolysis of starch : not hydrolyzed

- (4) Catalase : positive

- (5) Indole formation : not formed

- (6) Ammonia formation from peptone and arginine :  
negative

- (7) Methyl red test : negative

- (8) V-P test : negative

- (9) Attitude to oxygen : aerobic

- (10) O-F test (by the Hugh Leifson method): O type  
(Oxidation)

- (11) Acid formation from sugars
- positive : glucose, mannose, fructose and  
trehalose;

negative : arabinose, xylose, galactose,  
maltose, saccharose, sorbitol,  
lactose, inositol and glycerine

(12) Growth pH range : pH 6.0 - 9.0

5 (13) Optimum growth temperature : 25 - 37°C

The above bacteriological properties were examined with reference to the taxonomical standards in Bergey's Manual of Determinative Bacteriology, 7th edition (1957). As the result, J-4-2 strain was identified to be a strain belonging to the genus Pseudomonas from the various properties such as being a straight short-rod bacterium, Gram-negative, having polar flagella, having no spore-forming ability, being oxidative of glucose, etc. and was designated as Pseudomonas desmolytica J-4-2. The AT-6-7 strain, which is a short-rod bacterium almost approximate to a coccus, Gram-positive, forms no filament and forms acids from carbohydrates, was identified to be a strain belonging to the genus Brevibacterium and designated as Brevibacterium acetylicum AT-6-7. The BE-3-3 strain, which is Gram-negative, forms acids from hexose, forms hydrogen sulfide with its cells being rod-shaped, was identified to belong to the genus Achromobacter and designated as Achromobacter eurydice BE-3-3.

25 The above three microorganism strains were identified according to Bergey's Manual of Bacteriology, 7th edition, and it is possible that they may belong to other species

or genus when these strains are to be identified to belong to other species or genus according to different taxonomical standards due to some changes in taxonomical standards in the future. However, the microorganisms as designated above are inclusive of microorganisms which can at least produce nucleoside phosphorylase source in conformity with the object of the present invention and has the aforesaid bacteriological properties or bacteriological properties equivalent thereto, and can be unequivocally specified.

These three microorganism strains were deposited at the Fermentation Research Institute, Agency of Industrial Science & Technology on January 13, 1982, under the following deposition numbers (FERM P. No.). Further, these strains were sent directly from the Fermentation Research Institute to the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland, U.S.A. for deposition, and deposited on March 2, 1983, under the following deposition numbers (ATCC No.).

- 1) Pseudomonas desmolytica J-4-2, FERM P-6307,  
ATCC 39311
- 2) Brevibacterium acetylicum AT-6-7, FERM P-6305,  
ATCC 39311
- 3) Achromobacter eurydice BE-3-3, FERM P-6304,  
ATCC 39312

The mutant strains derived from the above micro-

organism strains through induced mutation according to the mutagenic methods in general by a physical treatment such as irradiation of UV-ray, X-ray or  $\gamma$ -ray or a chemical treatment with nitrosoguanidine or other mutagens or natural mutation attributable to natural causes may be also available in the present invention, so long as they do not lose the ability to produce nucleoside phosphorylase source suitable for the object of the present invention.

Further, when the gene for nucleoside phosphorylase source suitable for the object of the present invention of the microorganism strains preferably used in the present invention as described above is integrated in a microorganism other than the genera Pseudomonas, Brevibacterium, Achromobacter, Xanthomonas, Micrococcus, Staphylococcus and Sarcina if the characteristic of such a gene is phenotypically expressed, the method of employing the culture, the intact cells of such a microorganism or the modification thereof for the object of the present invention may also be included within the present invention.

In cultivation of these microorganisms to produce a nucleoside phosphorylase source, the culture medium and the method of culture employed are not particularly limited, so far as growth of these microorganisms is concerned.

As a culture medium, there may be employed one

containing appropriate amounts of a carbon source and a nitrogen source assimilable by these microorganisms, optionally added with an inorganic salt, minute amounts of a growth promoter, defoaming agents, etc. More specifically, as carbon sources, there may be employed one or more of those selected suitably in view of assimilability by the microorganism employed from carbon sources in general, including sugars such as glucose, fructose, maltose, galactose, ribose, saccharose, starch, starch hydrolysate, molasses, waste molasses, etc. or derivatives thereof such as fatty acid esters thereof; natural carbohydrates such as wheat, wheat bran, rice, etc.; alcohols such as glycerol, mannitol, methanol, ethanol, etc.; fatty acids such as gluconic acid, pyruvic acid, acetic acid, citric acid, etc.; hydrocarbons such as normal paraffins, kerosene, etc.; amino acids such as glycine, glutamic acid, glutamine, alanine, asparagine, etc.; and so on. As nitrogen sources, there may be employed one or more of those selected suitably in view of assimilability by the microorganism employed from nitrogen sources in general, including organic nitrogenous materials such as meat extract (bouillon), peptone, yeast extract, dry yeast, soybean hydrolysate, soybean powder, milk casein, casamino acid, various amino acids, corn steep liquor, cotton seed meal or its hydrolysate, fish meal or its hydrolysate, hydrolysates of other animals, vegetables,

microorganisms, etc.; inorganic nitrogen compounds such as ammonia, ammonium salts such as ammonium nitrate, ammonium sulfate, ammonium chloride, ammonium phosphate, ammonium carbonate, ammonium acetate and the like, nitric acid salts such as sodium nitrate, urea, and so on. Further, as inorganic salts, there may suitably be added one or more, in minute amounts, of phosphates, hydrochlorides, sulfates, carbonates, nitrates, acetates and others of magnesium, manganese, iron, zinc, copper, sodium, calcium, potassium, etc. If necessary, there may also be added a defoaming agent such as a vegetable oil or a surfactant, a minute amount of a growth promoter such as vitamins B<sub>1</sub>, B<sub>2</sub>, nicotinic acid, pantothenic acid, biotin, P-aminobenzoic acid, etc. When employing a microorganism exhibiting nutrient requirements, substances satisfying its growth must be added into the culture medium as a matter of course.

Cultivation may be performed in a liquid medium containing the above culture medium components by selecting a culture method suitable for the microorganism employed from conventional culture methods such as shaking culture, aerating stirring culture, stationary culture, continuous culture and others.

The cultural conditions may be suitably chosen depending on the microorganism and the culture medium employed, but generally by adjusting before start-up of cultivation at pH of about 6 to 8 and carrying out



cultivation under the temperature condition of about 25 to 35°C. The culture duration may be a period sufficient for growth of the microorganism employed, being generally 1 to 3 days.

5           After culturing the microorganism as described above, the culture, the intact microbial cells collected from the culture according to a conventional method such as centrifugation, sedimentation separation, agglomeration separation, or a modification of microbial cells obtained by applying a suitable treatment  
10           on the living or intact cells may be used as the nucleoside phosphorylase source of the present invention. The "culture" herein refers to a product under the state where the culture medium and the cultured microbial cells  
15           after cultivation are still unseparated from each other. The "modification of cells" refers to dried microbial cells, microbial cells whose cell wall membrane having been modified, crushed microbial cells, immobilized microbial cells, extracts of microbial cells, protein  
20           fractions having nucleoside phosphorylase activity of extract of microbial cells or purified product thereof, immobilized product of the protein fractions or purified product thereof, and the like. Methods for obtaining the modification of microbial cells are to be illustrated  
25           below. Modifications of microbial cells can be obtained by applying on intact microbial cells singly or in combination physical treatment means such as freezing-

thawing, lyophilization, air drying, acetone drying, heating under acidic or alkaline conditions, grinding, ultrasonic treatment, osmotic treatment, etc. or chemical or biochemical treatments such as enzymatic treatments with lysozyme, cell wall lysing enzymes, etc., contact treatments with solvents such as toluene, xylene, butyl alcohol or surfactants, or by applying on the extract of microbial cells singly or in combination enzyme separation and purification means such as salting-out, isoelectric precipitation, precipitation with organic solvents, various chromatographies, dialysis and others, or further by applying on intact microbial cells, extracts of microbial cells or purified products thereof an enzyme or cell immobilization means such as inclusion method, crosslinking method, adsorption method onto a carrier, etc.

#### Glycosylation or transglycosylation

The reaction in accordance with the present invention, namely enzymatic reaction of a guanine derivative of formula [I] with a 3-deoxyribose donor, which is glycosylation when the substituent R is hydrogen and is otherwise transglycosylation, is carried out by bringing a guanine derivative and a 3-deoxyribose donor into contact with the nucleoside phosphorylase source as described above in an aqueous medium. The kinds of the enzyme substrates are selected according to the kind of the enzyme source employed.

In a preferred embodiment of the present invention, there are two methods available to effect the contact.

The first method is one in which a guanine derivative and a 3-deoxyribose donor are caused to be present in the culture medium during cultivation of the aforesaid microorganism, thereby accumulating 3'-deoxyguanosine in the culture medium.

The above method may be carried out by adding necessary amounts of a guanine derivative and a 3-deoxyribose donor in the culture medium prior to cultivation and cultivating the microorganism therein, or by adding these substances at once at an appropriate period of time during cultivation, or by carrying out cultivation while adding intermittently or continuously these substances.

The second method may be carried out by bringing a culture, intact microbial cells or a modification of cells into contact with an aqueous medium containing a guanine derivative and a 3-deoxyribose donor under the conditions capable of forming 3'-deoxyguanosine.

An aqueous medium in which the contact between the reactants concerned is to take place may be water or various buffers preferred for enzymatic reactions (e.g. phosphate buffers, imidazole-hydrochloric acid buffer, veronal-hydrochloric acid buffer, Tris-hydrochloric acid buffer), which contains a phosphate ion generating source and may also contain various substances, if desired.

The enzymatic reaction of the present invention is mainly based on the action of phosphorylase, and therefore a phosphate ion must exist in the reaction system. In the case where a phosphate ion does not  
5 exist in the reaction system, an addition of a phosphate ion generating substance is necessary. As the phosphate ion generating substance, there may be employed any compound dissociable into phosphate ion in an aqueous medium, such as phosphoric acid itself,  
10 inorganic phosphoric acid salts such as salts of alkali metals, for example, sodium, potassium and the like, alkaline earth metals, for example, calcium, magnesium and the like or ammonium. These phosphate generating sources may be employed in amounts of about 1.0 to 2.5-  
15 fold moles per mole as phosphate ions per mole of the guanine derivative. As substances other than the phosphate ion generating source which may be contained in the aqueous medium, there may be employed sugars such as glucose, sacchrose and the like, organic solvents such  
20 as methanol, ethanol, propanol, butanol, pentanol, toluene, xylene, ethyl acetate and the like, various surfactants, metal salts and so on.

As the method to bring a nucleoside phosphorylase source into contact with a guanine derivative and a  
25 3-deoxyribose donor in an aqueous medium, there may be employed the method in which the nucleoside phosphorylase source is suspended or dissolved in an aqueous medium

containing these reaction substrates, optionally  
with stirring or shaking, or the method in which these  
reaction substrates are added at once, intermittently  
or continuously into a suspension or a solution of  
5 the nucleoside phosphorylase source in a reaction  
medium, or the method in which the nucleoside phos-  
phorylase source is packed in a column optionally  
admixed with a suitable diluent or carrier or immobiliz-  
ed onto a membrane and an aqueous medium containing the  
10 reaction substrates is passed therethrough.

During the reaction, the substrate concentration  
is not particularly limited, and the reaction may be  
carried out under a suspended state of the substrates.  
But each reaction substrate is used usually at a concent-  
15 ration within the range from 5 to 50 mM, preferably  
about 15 to 35 mM for a guanine derivative and about 15  
to 30 mM for 3-deoxyribose donor. The nucleoside  
phosphorylase source may be employed in an amount, which  
can easily be determined by those skilled in the art by  
20 considering the particular source material employed,  
the concentrations of the reaction substrates, the  
reaction efficiency and economy.

The reaction conditions, which are not particularly  
limited and may be determined while considering the  
25 optimum temperature and the optimum pH for the enzymatic  
action of the nucleoside phosphorylase source, stability  
of the substrates and reaction efficiency, may generally

comprise a temperature of 40 to 75°C, preferably 50 to 70°C and a pH 5.0 to 9.0, preferably 6.0 to 8.0. When pH is changed during the reaction, an acid or an alkali can be used to correct the pH to a preferred level. When a nucleoside phosphorylase source derived from the aforesaid three microorganism strains of genera Pseudomonas, Brevibacterium and Achromobacter, the optimum temperature is around 50 to 70°C and the reaction can be carried out at a relatively higher temperature, whereby there is the advantage that no countermeasure against microorganism contamination is required to be considered.

The reaction time, which may be determined while confirming the conversion of the reaction substrates to the desired product, may be generally about 15 to 45 hours, preferably 24 to 36 hours, in a batch system. In a columnar system, the reaction may be carried out under appropriate conditions set analogously as in the batch system.

After the enzymatic reaction, the nucleoside phosphorylase source may be removed by separation in a conventional manner, and the residual product is subjected to the step for isolation and purification of 3'-deoxyguanosine.

Isolation and purification of 3'-deoxyguanosine may be performed according to any of the methods known in the art by using separation/purification methods

singly or in combination such as various chromatographies, for example, ion-exchange chromatography, adsorption chromatography, partition chromatography, gel filtration, etc., the counter-current partition  
5 method, the recrystallization method and others.

Examples of the preferred embodiments

The present invention is to be described in further detail below by referring to Examples, each of which is illustrative of an embodiment of the present  
10 invention and not limitative of the scope of the present invention. In Examples, analysis of 3'-deoxyguanosine was conducted by high performance liquid chromatography. When analyzed by means of the device and under the conditions shown below, 3'-deoxyguanosine is eluted at a  
15 retention time around 12.90 minutes and its quantity can be calculated from the calibration curve.

Device: Shimadzu High Performance Liquid Chromatograph LC-3A model (produced by Shimadzu Corporation)

20 Column: Sorbax ODS, 4.6 mm x 250 mm (Shimadzu Du Pont Co.)

Eluant: 20 mM Tris-hydrochloric acid buffer containing 5% acetonitrile (pH 7.5)

Flow rate: 1 ml/minute

25 Column operation temperature: room temperature

Example A1

Two liters of a 2% bouillon culture medium were

sterilized at 120°C for 15 minutes and cooled. Then,  
100 ml of a previously precultured culture broth of  
Brevibacterium acetylicum AT-6-7 (FERM P-6305) was  
added to the culture broth and cultivation was carried  
5 out at 28°C for 22 hours.

After completion of the cultivation, the cells  
were collected by centrifugation and added into 200 ml  
of a sterilized water to be suspended therein. Into  
200 ml of a substrate solution (pH 7.0) containing 25  
10 mM GMP (disodium salt), 25 mM 3'-dAdo and 30 mM mono-  
sodium dihydrogen phosphate was added the above cell  
suspension and the reaction was carried out at 60°C  
for 36 hours.

The reaction mixture after removal of the cells by  
15 centrifugation was analyzed by high performance liquid  
chromatography to show that the yield of 3'-deoxyguano-  
sine was 36.58%. The yield of 3'-deoxyguanosine is  
defined as the molar ratio (%) of 3'-deoxyguanosine form-  
ed to 3'-deoxyadenosine added.

20 Example A2

Cultivation was carried out in the same manner as  
in Example A1 except that Pseudomonas desmolytica J-4-2  
(FERM P-6307) was used, and cells were collected and  
suspended in a sterilized water to obtain 200 ml of a  
25 cell suspension.

Into 200 ml of a substrate solution (pH 7.0)  
containing 25 mM Guo, 25 mM 3'-dAdo and 35 mM mono-



potassium dihydrogen phosphate was added the above cell suspension and the reaction was carried out at 60°C for 36 hours. The cells were removed by centrifugation and the reaction mixture was analyzed to show that the yield of 3'-deoxyguanosine was 42.09%. The reaction mixture was diluted to one liter (pH 9.0), treated with an anion exchange resin "Diaion SA-12A" (trade name; produced by Mitsubishi Kasei Kogyo Co., Ltd.) (borate form) and the solution which had passed through the column and the water washings were combined and adsorbed on a cation exchange resin "Diaion PK-216" (trade name; produced by Mitsubishi Kasei Kogyo Co., Ltd.) (free acid form), followed by elution. The fractions of 3'-deoxyguanosine were neutralized, concentrated and cooled. The crude crystals precipitated were recrystallized from hot water to obtain 449 mg of 3'-deoxyguanosine crystals.

#### Example A3

After cultivation of the same microorganism as in Example A2 conducted in the same manner except for using each 100 ml of a bouillon medium, cells were collected from each culture broth and 10 ml of a sterilized water was added to respective cells to prepare each cell suspension. To each of the suspensions was added each 10 ml of the solutions containing 20 mM 3'-dAdo, 25 mM monopotassium dihydrogen phosphate and 20 mM of the guanine derivative (Table A1), and the reaction was carried out at 60°C for 24 hours. After the reaction,

the supernatant obtained by centrifugation was analyzed to give the 3'-deoxyguanosine yield as shown in Table A1.

Table A1

5

Guanine derivative	3'-Deoxyguanosine yield (%)
Gua	2.14
Guo	26.36
GMP	21.72

10

When the same experiments were performed for Brevibacterium acetylicum AT-6-7 (FERM P-6305) and Achromobacter euridice BE-3-3 (FERM P-6304), the similar results as shown above were obtained with respect to the reactivities for guanine derivatives.

15

Example A4

Example A3 was repeated by use of Brevibacterium acetylicum AT-6-7 (FERM P-6305) as the nucleoside phosphorylase source, GMP as the guanine derivative and the respective enzymatic reaction temperatures of 40 to 80°C (Table A2), under otherwise the same conditions as in Example A3, and 3'-deoxyguanosines formed were analyzed to give the results as shown in Table A2.

20

25

Table A2

Reaction temperature	3'-Deoxyguanosine yield (%)
40°C	5.81
50°C	12.32
60°C	20.86
70°C	4.32
80°C	0

When the same experiments were performed for the BE-3-3 strain (FERM P-6304) and the J-4-2 strain (FERM P-6307), substantially the similar results were obtained with respect to the effect of the reaction temperature.

Example A5

Example A3 was repeated by use of Achromobacter euridice BE-3-3 (FERM P-6304) as the nucleoside phosphorylase source, GMP as the guanine derivative and the respective enzymatic reaction pH's of 6.0 to 9.0 (Table A2), under otherwise the same conditions as in Example A3, and 3'-deoxyguanosines formed were analyzed to give the results as shown in Table A3.

Table A3

Reaction pH	3'-Deoxyguanosine yield (%)
6.0	19.70
7.0	21.26
8.0	19.46
9.0	11.28

10           When the same experiments were performed for the AT-6-7 strain (FERM P-6305) and the J-4-2 strain (FERM P-6307), substantially the similar results were obtained with respect to the effect of pH.

Example A6

15           After cultivation was carried out in the same manner as in Example A1 by use of the same nucleoside phosphorylase source as in Example A5, the cells were collected and suspended in a sterilized water to obtain each 1 ml of cell suspensions. As the substrate solutions, there were prepared various combinations of aqueous solutions (pH 7.0) containing as a 3-deoxyribose donor 15 mM of 3'-dAdo, 3'-dAMP or 3'-dIno, as a guanine derivative 15 mM of GMP, GDP, GTP, 2'-dGuo or 2'-dGMP and 20 mM of monopotassium phosphate. Such cell suspensions and substrate solutions were mixed, respectively, and the reactions conducted at 60°C for 24 hours. As the result, the yields of 3'-deoxyguanosine obtained were as shown in Table A4.

Table A4

Substrate solution		3'-deoxyguano- sine yield (%)
3'-Deoxyribose donor	Guanine derivative	
5            3'-dAdo	GMP	62.70
3'-dAdo	GDP	56.02
3'-dAdo	GTP	14.87
3'-dAdo	2'-dGuo	43.82
3'-dAdo	2'-dGMP	34.50
<hr/>		
10           3'-dAMP	GMP	63.48
3'-dAMP	GDP	44.74
3'-dAMP	GTP	7.66
3'-dAMP	2'-dGuo	27.30
3'-dAMP	2'-dGMP	24.64
<hr/>		
15           3'-dIno	GMP	31.04

Example B1

One liter of a 2% bouillon culture medium were  
 20 sterilized at 120°C for 15 minutes and cooled. Then,  
 50 ml of a previously precultured culture broth of  
Staphylococcus aureus IAM 1011 was added to the culture  
 broth and cultivation was carried out at 28°C for 22  
 hours. After completion of the cultivation, the cells  
 25 were collected by centrifugation and a sterilized water  
 was added to prepare 100 ml of a cell suspension.

After 502.5 mg of 3'-dAdo, 849.7 mg of guanosine

and 272.0 mg of monopotassium dihydrogen phosphate were dissolved in a water under heating, the solution was made up to 100 ml (pH 7.0) and 100 ml of the above cell suspension was added thereto, followed by  
5 the reaction which was carried out at 55°C for 72 hours. After the reaction, the cells were removed by centrifugation and the reaction product was analyzed by high performance liquid chromatography to show that the yield of 3'-deoxyguanosine was 52.44%.

10 The reaction mixture after removal of the cells was diluted to 1000 ml (pH 9.0), treated with an anion exchange resin "Diaion SA-12A" (trade name; produced by Mitsubishi Kasei Kogyo Co., Ltd.) (borate form) and the solution which had passed through the column and  
15 the water washings were combined and adsorbed on a cation exchange resin "Diaion PK-216" (trade name; produced by Mitsubishi Kasei Kogyo Co., Ltd.) (free acid form), followed by elution, to obtain the fractions of 3'-deoxyguanosine. These fractions were neutralized,  
20 concentrated and cooled. The crude crystals precipitated were recrystallized from hot water to obtain 198.15 mg of 3'-deoxyguanosine crystals.

#### Example B2

The reactions were carried out by use of various  
25 guanine derivatives and 3'-dAdo or 3'-Ino as substrates and the same cell suspension as used in Example B1 as the enzyme source.

0090417

With the use of each 1 ml of an aqueous solution containing 20 mM 3-deoxyribose donor, 30 mM of a guanine derivative and 20 mM monopotassium dihydrogen phosphate, with addition of each 1 ml of the microbial cell suspension, the reaction was carried out at 55°C, at pH 7.0 for 70 hours. After the reaction, the microbial cells were removed by centrifugation from the reaction mixture, and the quantity of 3'-deoxyguanosine was measured and the yield was calculated to show the results as shown in Table B1.

Table B1

Substrate		3'-deoxyguanosine yield (%)
3-Deoxyribose donor	Guanine derivative	
3'-dAdo	Gua	25.4
	Guo	50.61
	GMP	34.57
	GDP	64.76
	GTP	34.55
	2'-dGuo	60.95
	2'-dGMP	72.30
3'-dAdo	Gua	8.37
	Guo	31.03
	GMP	22.11
	GDP	26.00
	GTP	13.22
	2'-dGuo	37.01
	2'-dGMP	32.96

Example B3

Various microorganisms as shown in Table B2 were cultured similarly as in Example B1, and the cultured microbial cells were collected to obtain cell suspensions similar to that in Example B1.

With the use of each 1 ml of an aqueous solution containing 20 mM of 3'-dAdo, 30 mM of GMP and 4 mM of monopotassium dihydrogen phosphate as the substrate solution, with addition of each 1 ml of the above cell suspensions, the reaction was carried out at 60°C, at pH 7.0, for 20 hours. The yields of 3'-deoxyguanosine formed were calculated similarly as described above to obtain the results as shown in Table B2.

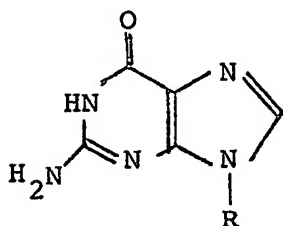
Table B2

Name of microorganism	3'-Deoxyguanosine yield (%)
<u>X. campestris</u> IAM 1671 (FERM P-6782)	9.82
<u>M. luteus</u> ATCC 4698	3.06
<u>St. aureus</u> IFO 3060	16.48
<u>St. epidermidis</u> IFO 3762 (ATCC 155)	15.02
<u>Sa. marginata</u> (FERM P-6539)	3.14



## WHAT IS CLAIMED IS:

1. A process for producing 3'-deoxyguanosine, which comprises causing a guanine derivative represented by the formula [I]:



[I]

wherein R designates a hydrogen atom or a ribose-1-yl group, a 2-deoxyribose-1-yl group or a monophosphate, a diphosphate or a triphosphate thereof, to react with a 3-deoxyribose donor in the presence of a nucleoside phosphorylase source to obtain 3'-deoxyguanosine.

2. A process for producing 3'-deoxyguanosine according to claim 1, wherein the 3-deoxyribose donor is selected from the group consisting of 3'-deoxyadenosine, 3'-deoxyinosine, and mono-, di- and triphosphates thereof and 3-deoxyribose-1-phosphate.

3. A process for producing 3'-deoxyguanosine according to claim 1 or 2, wherein the nucleoside phosphorylase source is of microorganism origin.

4. A process for producing 3'-deoxyguanosine

according to claim 3, wherein the nucleoside phosphorylase source is a culture, a mass of intact cells or a modification of cells of a microorganism which belongs to a genus Pseudomonas, Brevibacterium or Achromobacter and is capable of producing 3'-deoxyguanosine from the guanine derivative of the formula [I] and the 3-deoxyribose donor.

5. A process for producing 3'-deoxyguanosine according to claim 3, wherein the nucleoside phosphorylase source is a culture, a mass of intact cells or a modification of cells of a microorganism which belongs to a genus Xanthomonas, Micrococcus, Staphylococcus or Sarcina and is capable of producing 3'-deoxyguanosine from the guanine derivative of the formula [I] and the 3-deoxyribose donor.

6. A process for producing 3'-deoxyguanosine according to any of claims 1 to 5, wherein the guanine derivative is guanine, guanosine, 2'-deoxyguanosine, guanosine-5'-monophosphate, guanosine-5'-diphosphate, guanosine-5'-triphosphate or 2'-deoxyguanosine-5'-monophosphate.

⑫

**EUROPEAN PATENT SPECIFICATION**

- ④⑤ Date of publication of patent specification: **01.03.89**      ⑤① Int. Cl.<sup>4</sup>: **C 12 P 19/40 // C12N9/10**  
②① Application number: **83103128.1**  
②② Date of filing: **29.03.83**  
①① Divisional application **88100254** filed on **11.01.88**.

⑤④ **Process for producing 3'-deoxyguanosine.**

③① Priority: **01.04.82 JP 55078/82**  
**04.11.82 JP 194394/82**

④③ Date of publication of application:  
**05.10.83 Bulletin 83/40**

④⑤ Publication of the grant of the patent:  
**01.03.89 Bulletin 89/09**

⑧④ Designated Contracting States:  
**CH DE FR GB LI**

⑤① References cited:  
**EP-A-0 002 192**  
**FR-A-2 400 033**

**CHEMICAL ABSTRACTS, vol. 68, no. 3, 15th**  
**January 1968, page 916, no. 9666h, Columbus,**  
**Ohio, US. AKIRA IMADA et al.: "Ribosyl and**  
**deoxyribosyl transfer by bacterial enzyme**  
**systems" & J. BACTERIOL. 94(5), 1551-9, 1967**

**The file contains technical information**  
**submitted after th application was filed and**  
**not included in this specification**

⑦① Proprietor: **Yamasa Shoyu Kabushiki Kaisha**  
**10-1, Araoi-Cho 2-Chome**  
**Choshi-Shi Chiba-Ken 288 (JP)**

⑦② Inventor: **Fujishima, Tetsuro**  
**8539-8, Kobatake Shin Machi**  
**Choshi-Shi Chiba-Ken (JP)**  
Inventor: **Sakata, Shinji**  
**2-2, Sakae-Cho 2-Chome**  
**Choshi-Shi Chiba-Ken (JP)**

⑦④ Representative: **Dr. Elisabeth Jung Dr. Jürgen**  
**Schirdewahn Dipl.-Ing. Claus Gernhardt**  
**P.O. Box 40 14 68 Clemensstrasse 30**  
**D-8000 München 40 (DE)**

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European patent convention).

## Description

## Background of the Invention

This invention relates to an enzymatic process for producing 3'-deoxyguanosine.

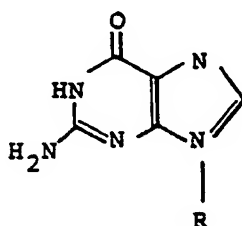
5 3'-Deoxyguanosine is a compound presently attracting attention which not only exhibits radiation sensitizing action in therapy of cancers but also has an action to enhance the effect of various anticancer agents when employed in combination therewith (see Japanese Laid-open Publication No. 35516/1982).

In the prior art, as the method for preparation 3'-deoxyguanosine, is known the method in which a chloromercuri of 2-acetamidohypoxanthine is condensed with 2,5-di-O-benzoyl-3-deoxy-D-ribofuranosyl bromide [The Journal of Organic Chemistry, 30, 2851 (1965)]. However, this method is believed to involve  
10 various drawbacks in commercial production such as, for example, difficult availability of the starting material 3-deoxyribose, formation of isomers during the condensation reaction, use of a harmful mercuric salt, and others.

## Summary of the Invention

The present inventors have made various investigations in order to overcome these drawbacks of the prior art and consequently found that 3'-deoxyguanosine can be formed by causing a guanine derivative to react with a 3-deoxyribose donor in the presence of a nucleoside phosphorylase source. The present invention has been accomplished based on such a finding.

20 The present invention provides a process for producing 3'-deoxyguanosine, which comprises causing a guanine derivative represented by the formula [I]:



[I]

wherein R designates a hydrogen atom or a ribose-1-yl group, a 2-deoxyribose-1-yl group or a monophosphate, a diphosphate or a triphosphate thereof, to react with a 3-deoxyribose donor in the  
35 presence of a nucleoside phosphorylase source to obtain 3'-deoxyguanosine.

## Detailed Description of the Invention

The present invention is to be described in detail below.

## 40 Guanine derivative

As the starting guanine derivatives, there may be employed one or more of the compounds included within the aforementioned definition. More specifically, illustrative of these compounds are guanine (Gua), guanosine (Guo) or 2'-deoxyguanosine (2'-dGuo) or monophosphates, diphosphates or triphosphates thereof. The above nucleotide may have a phosphoryl group at any position of hydroxyl groups in the  
45 sugar residue. Typical examples of these nucleotides may include guanosine-5'-monophosphate (GMP), guanosine-3'-monophosphate, guanosine-2'-monophosphate, guanosine-5'-diphosphate (GDP), guanosine-5'-triphosphate (GTP), 2'-deoxyguanosine-5'-monophosphate (2'-dGMP), 2'-deoxyguanosine-3'-monophosphate, 2'-deoxyguanosine-5'-diphosphate, (2'-dGDP), 2'-deoxyguanosine-5'-triphosphate (2'-dGTP) and the like, which may be either in free acid form or in an appropriate salt form such as sodium salt.

## 50 3-Deoxyribose donor

As the other starting material, a 3-deoxyribose donor, there may be employed any compounds which are capable of enzymatically introducing 3-deoxyribose moiety to the 9-position of the guanine moiety of the compound of formula [I] through direct glycosylation or transglycosylation. Typical examples include  
55 one or more of 3'-deoxyadenosine (3'-dAdo; cordycepin), 3'-deoxyinosine (3'-dIno), or monophosphates, diphosphates or triphosphates thereof, or 3-deoxyribose-1-monophosphate. The above phosphates may have phosphoryl group at any position of hydroxyl group in 3-deoxyribose, and may also be either in free acid form or in a salt form. Illustrative of these nucleotides are 3'-deoxyadenosine-5'-monophosphate (3'-dAMP), 3'-deoxyinosine-5'-monophosphate (3'-dIMP) or 3-deoxyribose-1-phosphate.

## 60 Nucleoside phosphorylase source

The nucleoside phosphorylase which is in the nucleoside phosphorylase source in the reaction of the present invention refers comprehensively to a single enzyme or a plurality of enzymes capable of providing  
65 3'-deoxyguanosine by causing the guanine derivative to react with the 3-deoxyribose donor in the presence of a phosphoric acid ion donor. Accordingly, in the present invention, the term "nucleoside

## EP 0 090 417 B1

phosphorylase source" includes the enzymes of the phosphorylase type such as purine nucleoside phosphorylase, pyrimidine nucleoside phosphorylase, etc., which can be used in combination with enzymes such as nucleoside-N-glycosyl transferase, nucleosidase, nucleotidase, phosphatase and others, which may possibly participate in the reaction of the present invention. The nucleoside phosphorylase source refers comprehensively to a material containing such enzymes in any desired form, which is not limited in its origin or source. That is, any enzyme material may be applicable so long as it can accomplish the object of the present invention, irrespective of whether it may be derived from microorganisms or from animals, or whether it may be prepared in any form. In particular, a nucleoside phosphorylase source of a microorganism origin, namely a nucleoside phosphorylase source in the form of a culture, a mass of intact cells or a modification of cells of a microorganism, is preferred.

Preferred nucleoside phosphorylase sources of microorganism origin are those based on microorganisms which belong to genera *Pseudomonas* (hereinafter referred to as Ps.), *Brevibacterium* (hereinafter referred to as Br.), *Achromobacter* (hereinafter referred to as Ach.), *Micrococcus* (hereinafter referred to as M.) and *Staphylococcus* hereinafter referred to as St.)

Typical strains of such microorganisms are:  
*Ps. desmolytica* J—4—2: FERM P—6307, ATCC 39310;  
*Br. acetylicum* AT—6—7: FERM P—6305, ATCC 39311;  
*Ach. eurydice* BE—3—3: FERM P—6304, ATCC 39312;

*M. luteus*: ATCC 4698, IAM 1056;  
*St. aureus*: IAM 1011, ATCC 6538P;  
*St. aureus*: IFO 3060;  
*St. epidermidis*: IFO 3762, ATCC 155;

Typical strains among these are the first three strains, namely J—4—2 strain isolated from the soil in Nishiashikajima-Cho, Choshi-Shi, Chiba-Ken, Japan; the AT—6—7 strain isolated from the sand in the baseball ground of Koshien, Nishinomiya-Shi, Hyogo-Ken, Japan; and the BE—3—3 strain isolated from the soil in Araoi-Cho, Choshi-Shi, Chiba-Ken, Japan. The bacteriological properties of these strains are set forth below.

### 1. J—4—2 strain

#### A. Morphology:

- (1) Form and size of cells: rod-shaped,  $0.7-0.8 \times 2.0-3.0 \mu\text{m}$ ;
- (2) Motility, occurrence of flagella, motile with polar flagella;
- (3) Formation of spores: none
- (4) Gram staining: negative

#### B. Growth on various culture media:

- (1) Bouillon-agar plate culture (28°C, 48 hours)
  - ① Form of colony: circular
  - ② Raising of colony surface: raised or umbonate
  - ③ Size: 2—4 mm
  - ④ Color tone: gray to buff when wet
- (2) Bouillon-agar slant culture (28°C, 48 hours)
  - ① Growth: good
  - ② Form of growth: filliform and slightly spreading
  - ③ Formation of pigment: not clear
- (3) Bouillon liquid culture (28°C, 72 hours)  
Growth: no membrane formation, turbid through-out the entire liquid, sediment slightly formed.
- (4) Bouillon-gelatin stab culture (20°C, 6 days): not liquefied
- (5) Litmus-milk culture medium (28°C, 4 days): not changed

#### C. Physiological properties

- (1) Reduction of a nitrate (28°C, 5 days): reductive
- (2) Formation of hydrogen sulfide (28°C, 5 days): formed
- (3) Hydrolysis of starch: not hydrolyzed
- (4) Catalase: positive
- (5) Indole formation: negative
- (6) Ammonia formation from p ptone and arginine: negativ
- (7) Methyl red test: n gative
- (8) V—P t st: positiv

**EP 0 090 417 B1**

- (9) Attitude to oxygen: aerobic
- (10) O—F test (by the Hugh Leifson method): O type (Oxidation)
- (11) Acid formation from sugars
  - positive: glucose, mannose, fructose, maltose, saccharose, trehalose and mannitol;
  - negative: arabinose, xylose, galactose, lactose, sorbitol, inositol and glycerine
- (12) Growth pH range: pH 6.0—9.0
- (13) Optimum growth temperature: 25—35°C

**2. AT—6—7 strain**

**10 A. Morphology:**

- (1) Form and size of cells: short rod-shaped, 0.8—1.0 × 1.0—1.2 µm;
- (2) Formation of spores: none
- (4) Gram staining: negative

**15 B. Growth on various culture media:**

**(1) Bouillon-agar plate culture (28°C, 48 hours)**

- ① Form of colony: circular
- ② Raising of colony surface: flat, smooth
- ③ Size: 2—4 mm
- ④ Color tone: yellow to peach-yellow

**(2) Bouillon-agar slant culture (28°C, 48 hours)**

- ① Growth: good
- ② Form of growth: echinulate

**(3) Bouillon liquid culture (28°C, 48 hours)**

Growth: formation of ring on the surface, sediment slightly formed.

**(4) Bouillon-gelatin stab culture (20°C, 6 days): liquefied in stratiform**

**(5) Litmus-milk culture medium (28°C, 4 days): slightly coagulated, peptonization also observed**

**C. Physiological properties**

- (1) Reduction of a nitrate (28°C, 5 days): no reductivity
- (2) Formation of hydrogen sulfide (28°C, 5 days): not formed
- (3) Hydrolysis of starch: hydrolyzed
- (4) Catalase: positive
- (5) Indole formation: not formed
- (6) Ammonia formation from peptone and arginine: negative
- (7) Methyl red test: negative
- (8) V—P test: positive
- (9) Attitude to oxygen: aerobic
- (10) O—F test (by the Hugh Leifson method): F type (Fermentation)
- (11) Acid formation from sugars
  - positive: glucose, mannose, fructose, maltose, saccharose and trehalose;
  - negative: arabinose, xylose, galactose, lactose, sorbitol, inositol and glycerine
- (12) Growth pH range: pH 6.0—9.0
- (13) Optimum growth temperature: 25—37°C

**3. BE—3—3 strain**

**A. Morphology**

- (1) Form and size of cells: rod-shaped, 0.8—0.9 × 1.4—1.8 µm;
- (2) Formation of spores: none
- (4) Gram staining: negative

**B. Growth on various culture media:**

**(1) Bouillon-agar plate culture (28°C, 48 hours)**

- ① Form of colony: rhizoid and lacerate
- ② Raising of colony surface: flat and smooth
- ③ Size: 5—9 mm
- ④ Color tone: pale grayish brown to bluish gray

**(2) Bouillon-agar slant culture (28°C, 48 hours)**

- ① Growth: good
- ② Form of growth: filiform

## EP 0 090 417 B1

(3) Bouillon liquid culture (28°C, 48 hours)  
Growth: turbid throughout the entire liquid, sediment formed.

(4) Bouillon-gelatin stab culture (20°C, 6 days): not liquefied

(5) Litmus-milk culture medium (28°C, 4 days): substantially not changed

### C. Physiological properties

- (1) Reduction of a nitrate (28°C, 5 days): no reductivity
- (2) Formation of hydrogen sulfide (28°C, 5 days): formed
- (3) Hydrolysis of starch: not hydrolyzed
- (4) Catalase: positive
- (5) Indole formation: not formed
- (6) Ammonia formation from peptone and arginine: negative
- (7) Methyl red test: negative
- (8) V—P test: negative
- (9) Attitude to oxygen: aerobic
- (10) O—F test (by the Hugh Leifson method): O type (Oxidation)
- (11) Acid formation from sugars  
positive: glucose, mannose, fructose and trehalose;  
negative: arabinose, xylose, galactose, maltose, saccharose, sorbitol, lactose, inositol and glycerine
- (12) Growth pH range: pH 6.0—9.0
- (13) Optimum growth temperature: 25—37°C

The above bacteriological properties were examined with reference to the taxonomical standards in Bergey's Manual of Determinative Bacteriology, 7th edition (1957). As the result, J—4—2 strain was identified to be a strain belonging to the genus *Pseudomonas* from the various properties such as being a straight short-rod bacterium, Gram-negative, having polar flagella, having no spore-forming ability, being oxidative of glucose, etc. and was designated as *Pseudomonas desmolytica* J—4—2. The AT—6—7 strain, which is a short-rod bacterium almost approximate to a coccus, Gram-positive, forms no filament and forms acids from carbohydrates, was identified to be a strain belonging to the genus *Brevibacterium* and designated as *Brevibacterium acetylicum* AT—6—7. The BE—3—3 strain, which is Gram-negative, forms acids from hexose, forms hydrogen sulfide with its cells being rod-shaped, was identified to belong to the genus *Achromobacter* and designated as *Achromobacter eurydice* BE—3—3.

The above three microorganism strains were identified according to Bergey's Manual of Bacteriology, 7th edition, and it is possible that they may belong to other species or genus when these strains are to be identified to belong to other species or genus according to different taxonomical standards due to some changes in taxonomical standards in the future. However, the microorganisms as designated above are inclusive of microorganisms which can at least produce nucleoside phosphorylase source in conformity with the object of the present invention and has the aforesaid bacteriological properties or bacteriological properties equivalent thereto, and can be unequivocally specified.

These three microorganism strains were deposited at the Fermentation Research Institute, Agency of Industrial Science & Technology on January 13, 1982, under the following deposition numbers (FERM P. No.). Further, these strains were sent directly from the Fermentation Research Institute of the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland, U.S.A. for deposition, and deposited on March 2, 1983, under the following deposition numbers (ATCC No.).

- 1) *Pseudomonas desmolytica* J—4—2, FERM P—6307, ATCC 39311
- 2) *Brevibacterium acetylicum* AT—6—7, FERM P—6305, ATCC 39311
- 3) *Achromobacter eurydice* BE—3—3, FERM P—6304, ATCC 39312

The mutant strains derived from the above microorganism strains through induced mutation according to the mutagenic methods in general by a physical treatment such as irradiation of UV-ray, X-ray or  $\gamma$ -ray or a chemical treatment with nitrosoguanidine or other mutagens or natural mutation attributable to natural causes may be also available in the present invention, so long as they do not lose the ability to produce nucleoside phosphorylase source suitable for the object of the present invention.

Further, when the gene for nucleoside phosphorylase source suitable for the object of the present invention of the microorganism strains preferably used in the present invention as described above is integrated in a microorganism other than the genera *Pseudomonas*, *Brevibacterium*, *Achromobacter*, *Micrococcus* and *Staphylococcus* if the characteristic of such a gene is phenotypically expressed, the method of employing the culture, the intact cells of such a microorganism or the modification thereof for the object of the present invention may also be included within the present invention.

In cultivation of these microorganisms to produce a nucleoside phosphorylase source, the culture medium and the method of culture employed are not particularly limited, so far as growth of these microorganisms is concerned.

As a culture medium, there may be employed one containing appropriate amounts of a carbon source

## EP 0 090 417 B1

and a nitrogen source assimilable by these microorganisms, optionally added with an inorganic salt, minute amounts of a growth promoter, defoaming agents, etc. More specifically, as carbon sources, there may be employed one or more of those selected suitably in view of assimilability by the microorganism employed from carbon sources in general, including sugars such as glucose, fructose, maltose, galactose, 5 ribose, saccharose, starch, starch hydrolysate, molasses, waste molasses, etc. or derivatives thereof such as fatty acid esters thereof; natural carbohydrates such as wheat, wheat bran, rice, etc.; alcohols such as glycerol, mannitol, methanol, ethanol, etc.; fatty acids such as gluconic acid, pyruvic acid, acetic acid, citric acid, etc.; hydrocarbons such as normal paraffins, kerosene, etc.; amino acids such as glycine, glutamic acid, glutamine, alanine, asparagine, etc.; and so on. As nitrogen sources, there may be employed one or 10 more of those selected suitably in view of assimilability by the microorganism employed from nitrogen sources in general, including organic nitrogenous materials such as meat extract (bouillon), peptone, yeast extract, dry yeast, soybean hydrolysate, soybean powder, milk casein, casamino acid, various amino acids, corn steep liquor, cotton seed meal or its hydrolysate, fish meal or its hydrolysate, hydrolysates of other animals, vegetables, microorganisms, etc.; inorganic nitrogen compounds such as ammonia, ammonium 15 salts such as ammonium nitrate, ammonium sulfate, ammonium chloride, ammonium phosphate, ammonium carbonate, ammonium acetate and the like, nitric acid salts such as sodium nitrate, urea, and so on. Further, as inorganic salts, there may suitably be added one or more, in minute amounts, of phosphates, hydrochlorides, sulfates, carbonates, nitrates, acetates and others of magnesium, manganese, iron, zinc, copper, sodium, calcium, potassium etc. If necessary, there may also be added a defoaming 20 agent such as a vegetable oil or a surfactant, a minute amount of a growth promoter such as vitamins B<sub>1</sub>, B<sub>2</sub>, nicotinic acid, pantothenic acid, biotin, P-aminobenzoic acid, etc. When employing a microorganism exhibiting nutrient requirements, substances satisfying its growth must be added into the culture medium as a matter of course.

Cultivation may be performed in a liquid medium containing the above culture medium components 25 by selecting a culture suitable for the microorganism employed from conventional culture methods such as shaking culture, aerating stirring culture, stationary culture, continuous culture and others.

The cultural conditions may be suitably chosen depending on the microorganism and the culture medium employed, but generally by adjusting before start-up of cultivation at pH of about 6 to 8 and carrying out cultivation under the temperature condition of about 25 to 35°C. The culture duration may be a 30 period sufficient for growth of the microorganism employed, being generally 1 to 3 days.

After culturing the microorganism as described above, the culture, the intact microbial cells collected from the culture according to a conventional method such as centrifugation, sedimentation separation, agglomeration separation, or a modification of microbial cells obtained by applying a suitable treatment on 35 the living or intact cells may be used as the nucleoside phosphorylase source of the present invention. The "culture" herein refers to a product under the state where the culture medium and the cultured microbial cells after cultivation are still unseparated from each other. The "modification of cells" refers to dried microbial cells, microbial cells whose cell wall membrane having been modified, crushed microbial cells, immobilized microbial cells, extracts of microbial cells, protein fractions having nucleoside phosphorylase activity of extract of microbial cells or purified product thereof, immobilized product of the protein fractions 40 or purified product thereof, and the like. Methods for obtaining the modification of microbial cells are to be illustrated below. Modification of microbial cells can be obtained by applying on intact microbial cells singly or in combination physical treatment means such as freezing, thawing, lyophilization, air drying, acetone drying, heating under acidic or alkaline conditions, grinding, ultrasonic treatment, osmotic treatment, etc. or chemical or biochemical treatments such as enzymatic treatments with lysozyme, cell 45 wall lysing enzymes, etc., contact treatments with solvents such as toluene, xylene, butyl alcohol or surfactants, or by applying on the extract of microbial cells singly or in combination enzyme separation and purification means such as salting-out, isoelectric precipitation, precipitation with organic solvents, various chromatographies, dialysis and others, or further applying on intact microbial cells, extracts of microbial cells or purified products thereof an enzyme or cell immobilization means such as inclusion method, 50 crosslinking method, adsorption method onto a carrier etc.

### Glycosylation or transglycosylation

The reaction in accordance with the present invention, namely enzymatic reaction of a guanine derivative of formula [I] with a 3-deoxyribose donor, which is glycosylation when the substituent R is 55 hydrogen and is otherwise transglycosylation, is carried out by bringing a guanine derivative and a 3-deoxyribose donor into contact with the nucleoside phosphorylase source as described above in an aqueous medium. The kinds of the enzyme substrates are selected according to the kind of the enzyme source employed.

In a preferred embodiment of the present invention, there are two methods available to effect the 60 contact.

The first method is one in which a guanine derivative and a 3-deoxyribose donor are caused to be present in the culture medium during cultivation of the afor said microorganism, thereby accumulating 3'-deoxyguanosine in the culture medium.

The above method may be carried out by adding necessary amounts of a guanine derivative and a 3- 65 deoxyribose donor in the culture medium prior to cultivation and cultivating the microorganism therein, or



by adding these substances at once at an appropriate period of time during cultivation, or by carrying out cultivation while adding intermittently or continuously these substances.

The second method may be carried out by bringing a culture, intact microbial cells or a modification of cells into contact with an aqueous medium containing a guanine derivative and a 3'-deoxyribose donor under the conditions capable of forming 3'-deoxyguanosine.

An aqueous medium in which the contact between the reactants concerned is to take place may be water or various buffers preferred for enzymatic reactions (e.g. phosphate buffers, imidazole-hydrochloric acid buffer, veronal-hydrochloric acid buffer, Tris-hydrochloric acid buffer), which contains a phosphate ion generating source and may also contain various substances, if desired.

The enzymatic reaction of the present invention is mainly based on the action of phosphorylase, and therefore a phosphate ion must exist in the reaction system. In the case where a phosphate ion does not exist in the reaction system, an addition of a phosphate ion generating substance is necessary. As the phosphate ion generating substance, there may be employed any compound dissociable into phosphate ion in an aqueous medium, such as phosphoric acid itself, inorganic phosphoric acid salts such as salts of alkali metals, for example, sodium, potassium and the like, alkaline earth metals, for example, calcium, magnesium and the like or ammonium. These phosphate generating sources may be employed in amounts of about 1.0 to 2.5-fold moles per mole as phosphate ions per mole of the guanine derivative. As substances other than the phosphate ion generating source which may be contained in the aqueous medium, there may be employed sugars such as glucose, saccharose and the like, organic solvents such as methanol, ethanol, propanol, butanol, pentanol, toluene, xylene, ethyl acetate and the like, various surfactants, metals salts and so on.

As the method to bring a nucleoside phosphorylase source into contact with a guanine derivative and a 3'-deoxyribose donor in an aqueous medium, there may be employed the method in which the nucleoside phosphorylase source is suspended or dissolved in an aqueous medium containing these reaction substrates, optionally with stirring or shaking, or the method in which these reaction substrates are added at once, intermittently or continuously into a suspension or a solution of the nucleoside phosphorylase source in a reaction medium, or the method in which the nucleoside phosphorylase source is packed in a column optionally admixed with a suitable diluent or carrier or immobilized onto a membrane and an aqueous medium containing the reaction substrates is passed therethrough.

During the reaction, the substrate concentration is not particularly limited, and the reaction may be carried out under a suspended state of the substrates. But each reaction substrate is used usually at a concentration within the range from 5 to 50 mM, preferably about 15 to 35 mM for a guanine derivative and about 15 to 30 mM for 3'-deoxyribose donor. The nucleoside phosphorylase source may be employed in an amount, which can easily be determined by those skilled in the art by considering the particular source material employed, the concentrations of the reaction substrates, the reaction efficiency and economy.

The reaction conditions, which are not particularly limited and may be determined while considering the optimum temperature and the optimum pH for the enzymatic action of the nucleoside phosphorylase source, stability of the substrates and reaction efficiency, may generally comprise a temperature of 40 to 75°C, preferably 50 to 70°C and a pH 5.0 to 9.0, preferably 6.0 to 8.0. When pH is changed during the reaction, an acid or an alkali can be used to correct the pH to a preferred level. When a nucleoside phosphorylase source derived from the aforesaid three microorganism strains of genera *Pseudomonas*, *Brevibacterium* and *Achromobacter*, the optimum temperature is around 50 to 70°C and the reaction can be carried out at a relatively higher temperature, whereby there is the advantage that no countermeasure against microorganism contamination is required to be considered.

The reaction time, which may be determined while confirming the conversion of the reaction substrates to the desired product, may be generally about 15 to 45 hours, preferably 24 to 36 hours, in a batch system. In a columnar system, the reaction may be carried out under appropriate conditions set analogously as in the batch system.

After the enzymatic reaction, the nucleoside phosphorylase source may be removed by separation in a conventional manner, and the residual product is subjected to the step for isolation and purification of 3'-deoxyguanosine.

Isolation and purification of 3'-deoxyguanosine may be performed according to any of the methods known in the art by using separation/purification methods singly or in combination such as various chromatographies, for example, ion-exchange chromatography, adsorption chromatography, partition chromatography, gel filtration, etc., the counter-current partition method, the recrystallization method and others.

#### Examples of the preferred embodiments

The present invention is to be described in further detail below by referring to Examples, each of which is illustrative of an embodiment of the present invention and not limitative of the scope of the present invention. In Examples, analysis of 3'-deoxyguanosine was conducted by high performance liquid chromatography. When analyzed by means of the device and under the conditions shown below, 3'-deoxyguanosine is eluted at a retention time around 12.90 minutes and its quantity can be calculated from the calibration curve.

## EP 0 090 417 B1

Device: Shimadzu High Performance Liquid Chromatograph LC—3A model (produced by Shimadzu Corporation)

Column: Sorbax ODS, 4.6 mm × 250 mm (Shimadzu Du Pont Co.)

Eluant: 20 mM Tris-hydrochloric acid buffer containing 5% acetonitrile (pH 7.5)

Flow rate: 1 ml/minute

Column operation temperature: room temperature.

### Example A1

Two liters of a 2% bouillon culture medium were sterilized at 120°C for 15 minutes and cooled. Then, 100 ml of a previously precultured culture broth of *Brevibacterium acetylicum* AT—6—7 (FERM P—6305) was added to the culture broth and cultivation was carried out at 28°C for 22 hours.

After completion of the cultivation, the cells were collected by centrifugation and added into 200 ml of a sterilized water to be suspended therein. Into 200 ml of a substrate solution (pH 7.0) containing 25 mM GMP (disodium salt), 25 mM 3'-dAdo and 30 mM monosodium dihydrogen phosphate was added the above cell suspension and the reaction was carried out at 60°C for 36 hours.

The reaction mixture after removal of the cells by centrifugation was analyzed by high performance liquid chromatography to show that the yield of 3'-deoxyguanosine was 36.58%. The yield of 3'-deoxyguanosine is defined as the molar ratio (%) of 3'-deoxyguanosine formed to 3'-deoxyadenosine added.

### Example A2

Cultivation was carried out in the same manner as in Example A1 except that *Pseudomonas desmolytica* J—4—2 (FERM P—6307) was used, and cells were collected and suspended in a sterilized water to obtain 200 ml of a cell suspension.

Into 200 ml of substrate solution (pH 7.0) containing 25 mM Guo 25 mM 3'-dAdo and 35 mM monopotassium dihydrogen phosphate was added the above cell suspension and the reaction was carried out at 60°C for 36 hours. The cells were removed by centrifugation and the reaction mixture was analyzed to show that the yield of 3'-deoxyguanosine was 42.09%. The reaction mixture was diluted to one liter (pH 9.0), treated with an anion exchange resin "Diaion SA—12A" (trade name; produced by Mitsubishi Kasei Kogyo Co., Ltd.) (borate form) and the solution which had passed through the column and the water washings were combined and adsorbed on a cation exchange resin "Diaion PK—216" (trade name; produced by Mitsubishi Kasei Kogyo Co., Ltd.) (free acid form), followed by elution. The fractions of 3'-deoxyguanosine were neutralized, concentrated and cooled. The crude crystals precipitated were recrystallized from hot water to obtain 449 mg of 3'-deoxyguanosine crystals.

### Example A3

After cultivation of the same microorganism as in Example A2 conducted in the same manner except for using each 100 ml of bouillon medium, cells were collected from each culture broth and 10 ml of a sterilized water was added to respective cells to prepare each cell suspension. To each of the suspensions was added each 10 ml of the solutions containing 20 mM 3'-dAdo, 25 mM monopotassium dihydrogen phosphate and 20 mM of the guanine derivative (Table A1), and the reaction was carried out at 60°C for 24 hours. After the reaction, the supernatant obtained by centrifugation was analyzed to give the 3'-deoxyguanosine yield as shown in Table A1.

TABLE A1

Guanine derivative	3'-Deoxyguanosine yield (%)
Gua	2.14
Guo	26.36
GMP	21.72

When the same experiments were performed for *Brevibacterium acetylicum* AT—6—7 (FERM P—6305) and *Achromobacter euidice* BE—3—3 (FERM P—6304), the similar results as shown above were obtained with respect to the reactivities for guanine derivatives.

### Example A4

Example A3 was repeated by use of *Brevibacterium acetylicum* AT—6—7 (FERM P—6305) as the nucleoside phosphorylase source, GMP as the guanine derivative and the respective enzymatic reaction temperatures of 40 to 80°C (Table A2), under otherwise the same conditions as in Example A3, and 3'-deoxyguanosines formed were analyzed to give the results as shown in Table A2.

# EP 0 090 417 B1

TABLE A2

Reaction temperature	3'-Deoxyguanosine yield (%)
40°C	5.81
50°C	12.32
60°C	20.86
70°C	4.32
80°C	0

When the same experiments were performed for the BE—3—3 strain (FERM P—6304) and the J—4—2 strain (FERM P—6307), substantially the similar results were obtained with respect to the effect of the reaction temperature.

## Example A5

Example A3 was repeated by use of *Achromobacter euidice* BE—3—3 (FERM P—6304) as the nucleoside phosphorylase source, GMP as the guanine derivative and the respective enzymatic reaction pH's of 6.0 to 9.0 (Table A2), under otherwise the same conditions as in Example A3, and 3'-deoxyguanosines formed were analyzed to give the results as shown in Table A3.

TABLE A3

Reaction pH	3'-Deoxyguanosine yield (%)
6.0	19.70
7.0	21.26
8.0	19.46
9.0	11.28

When the same experiments were performed for the AT—6—7 strain (FERM P—6305) and the J—4—2 strain (FERM P—6307), substantially the similar results were obtained with respect to the effect of pH.

## Example A6

After cultivation was carried out in the same manner as in Example A1 by use of the same nucleoside phosphorylase source as in Example A5, the cells were collected and suspended in a sterilized water to obtain each 1 ml of cell suspensions. As the substrate solutions, there were prepared various combinations of aqueous solutions (Ph 7.0) containing as a 3-deoxyribose donor 15 mM of 3'-dAdo, 3'-dAMP or 3'-dIno, as a guanine derivative of 15 mM of GMP, GDP, GTP, 2'-dGuo or 2'-dGMP and 20 mM of monopotassium phosphate. Such cell suspensions and substrate solutions were mixed, respectively, and the reactions conducted at 60°C for 24 hours. As the result, the yields of 3'-deoxyguanosine obtained were as shown in Table A4.

TABLE A4

Substrate solution		3'-deoxyguanosine yield %
3'-Deoxyribose donor	Guanine derivative	
3'-dAdo	GMP	62.70
3'-dAdo	GDP	56.02
3'-dAdo	GTP	14.87
3'-dAdo	2'-dGuo	43.82
3'-dAdo	2'-dGMP	34.50
3'-dAMP	GMP	63.48
3'-dAMP	GDP	44.74
3'-dAMP	GTP	7.66
3'-dAMP	2'-dGuo	27.30
3'-dAMP	2'-dGMP	24.64
3'-dIno	GMP	31.04

# EP 0 090 417 B1

## Example B1

One liter of a 2% bouillon culture medium were sterilized at 120°C for 15 minutes and cooled. Then, 50 ml of a previously precultured culture broth of *Staphylococcus aureus* IAM 1011 was added to the culture broth and cultivation was carried out at 28°C for 22 hours. After completion of the cultivation, the cells were collected by centrifugation and a sterilized water was added to prepare 100 ml of a cell suspension.

After 502.5 mg of 3'-dAdo, 849.7 mg of guanosine and 272.0 mg of monopotassium dihydrogen phosphate were dissolved in a water under heating, the solution was made up to 100 ml (pH 7.0) and 100 ml of the above cell suspension was added thereto, followed by the reaction which was carried out at 55°C for 72 hours. After the reaction, the cells were removed by centrifugation and the reaction product was analyzed by high performance liquid chromatography to show that the yield of 3'-deoxyguanosine was 52.44%.

The reaction mixture after removal of the cells was diluted to 1000 ml (pH 9.0), treated with an anion exchange resin "Diaion SA-12A" (trade name; produced by Mitsubishi Kasei Kogyo Co., Ltd.) (borate form) and the solution which had passed through the column and the water washings were combined and adsorbed on a cation exchange resin "Diaion PK-216" (trade name; produced by Mitsubishi Kasei Kogyo Co., Ltd.) (free acid form), followed by elution, to obtain the fractions of 3'-deoxyguanosine. These fractions were neutralized, concentrated and cooled. The crude crystals precipitated were recrystallized from hot water to obtain 198.15 mg of 3'-deoxyguanosine crystals.

## Example B2

The reactions were carried out by use of various guanine derivatives and 3'-dAdo or 3'-Ino as substrates and the same cell suspension as used in Example B1 as the enzyme source.

With the use of each 1 ml of an aqueous solution containing 20 mM deoxyribose donor, 30 mM of a guanine derivative and 20 mM monopotassium dihydrogen phosphate, with addition of each 1 ml of the microbial cell suspension, the reaction was carried out at 55°C, at pH 7.0 for 70 hours. After the reaction, the microbial cells were removed by centrifugation from the reaction mixture, and the quantity of 3'-deoxyguanosine was measured and the yield was calculated to show the results as shown in Table B1.

TABLE B1

Substrate		3'-deoxyguanosine yield %
3'-Deoxyribose donor	Guanine derivative	
3'-dAdo	Gua	25.4
	Guo	50.61
	GMP	34.57
	GDP	64.76
	GTP	34.55
	2'-dGuo	60.95
	2'-dGMP	72.30
3'-dAdo	Gua	8.37
	Guo	31.03
	GMP	22.11
	GDP	26.00
	GTP	13.22
	2'-dGuo	37.01
	2'-dGMP	32.96

## Example B3

Various microorganisms as shown in Table B2 were cultured similarly as in Example B1, and the cultured microbial cells were collected to obtain cell suspensions similar to that in Example B1.

With the use of each 1 ml of an aqueous solution containing 20 mM of 3'-dAdo, 30 mM of GMP and 4 mM of monopotassium dihydrogen phosphate as the substrate solution, with addition of each 1 ml of the above cell suspensions, the reaction was carried out at 60°C, at pH 7.0, for 20 hours. The yields of 3'-deoxyguanosine formed were calculated similarly as described above to obtain the results as shown in Table B2.

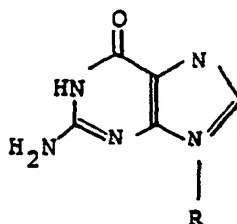
# EP 0 090 417 B1

TABLE B2

Name of microorganism	3'-Deoxyguanosine yield (%)
<i>M. luteus</i> ATCC 4698	3.06
<i>St. aureus</i> IFO 3060	16.48
<i>St. epidermidis</i> IFO 3762 (ATCC 155)	15.02

## Claims

1. A process for producing 3'-deoxyguanosine, which comprises a guanine derivative represented by the formula [I]:



wherein R designates a hydrogen atom or a ribose-1-yl group, a 2-deoxyribose-1-yl group or a monophosphate, a diphosphate or a triphosphate thereof, to react with a 3-deoxyribose donor in the presence of a nucleoside phosphorylase source to obtain 3'-deoxyguanosine.

2. A process for producing 3'-deoxyguanosine according to claim 1, wherein the 3-deoxyribose donor is selected from the group consisting of 3'-deoxyadenosine, 3'-deoxyinosine, and mono-, di- and triphosphates thereof and 3-deoxyribose-1-phosphate.

3. A process for producing 3'-deoxyguanosine according to claim 1 or 2, wherein the nucleoside phosphorylase source is of microorganism origin.

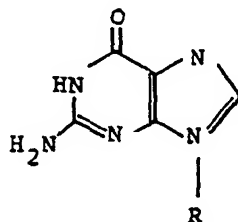
4. A process for producing 3'-deoxyguanosine according to claim 3, wherein the nucleoside phosphorylase source is a culture, a mass of intact cells or a modification of cells of a microorganism which belongs to a genus *Pseudomonas*, *Brevibacterium* or *Achromobacter* and is capable of producing 3'-deoxyguanosine from the guanine derivative of the formula [I] and the deoxyribose donor.

5. A process for producing 3'-deoxyguanosine according to claim 3, wherein the nucleoside phosphorylase source is a culture, a mass of intact cells or a modification of cells of a microorganism which belongs to *Micrococcus* or *Staphylococcus* and is capable of producing 3'-deoxyguanosine from the guanine derivative of the formula [I] and the 3-deoxyribose donor.

6. A process for producing 3'-deoxyguanosine according to any of claims 1 to 5, wherein the guanine derivative is guanine, guanosine, 2'-deoxyguanosine, guanosine-5'-monophosphate, guanosine-5'-diphosphate, guanosine-5'-triphosphate or 2'-deoxyguanosine-5'-monophosphate.

## Patentansprüche

1. Ein Verfahren zur Herstellung von 3'-Desoxyguanosin, in welchem man ein Guandinderivat, dargestellt durch die Formel (I):



in welcher R ein Wasserstoffatom oder eine Ribose-1-yl-Gruppe, eine 2-Desoxyribose-1-yl-Gruppe oder ein Monophosphat, eine Diphosphat oder ein Triphosphat davon bedeutet, mit einem 3-Desoxyribosidonator in Gegenwart einer Nucleosidphosphorylasequelle zu 3'-Desoxyguanosin reagieren läßt.

2. Ein Verfahren zur Herstellung von 3'-Desoxyguanosin nach Anspruch 1, in welchem der 3-Desoxyribosidonator ausgewählt ist aus der Gruppe bestehend aus 3'-Desoxyadenosin, 3'-Desoxyinosin, und Mono-, Di- und Triphosphaten davon und 3-Desoxyribose-1-phosphat.

3. Ein Verfahren zur Herstellung von 3'-Desoxyguanosin nach Anspruch 1 oder 2, in welchem die Nukleosidphosphorylasequelle von einem Mikroorganismus stammt.

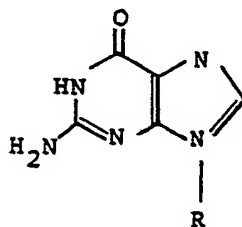
4. Ein Verfahren zur Herstellung von 3'-Desoxyguanosin nach Anspruch 3, in welchem die Nukleosidphosphorylasequelle eine Kultur, eine Masse aus intakten Zellen oder eine Modifikation von Zellen eines Mikroorganismus ist, welcher zur Gattung *Pseudomonas*, *Brevibacterium* oder *Achromobacter* gehört und dazu in der Lage ist, 3'-Desoxyguanosin aus dem Guaninderivat der Formel (I) und dem 3-Desoxyribosedonator herzustellen.

5. Ein Verfahren zur Herstellung von 3'-Desoxyguanosin nach Anspruch 3, in welchem die Nukleosidphosphorylasequelle eine Kultur, eine Masse aus intakten Zellen oder eine Modifikation von Zellen eines Mikroorganismus ist, welcher zur Gattung *Micrococcus* oder *Staphylococcus* gehört und dazu fähig ist, 3'-Desoxyguanosin aus dem Guaninderivat der Formel (I) und dem 3-Desoxyribosedonator herzustellen.

6. Ein Verfahren zur Herstellung von 3'-Desoxyguanosin nach einem der Ansprüche 1 bis 5, in welchem das Guaninderivat Guanin, Guanosin, 2'-Desoxyguanosin, Guanosin-5'-monophosphat, Guanosin-5'-diphosphat, Guanosin-5'-triphosphat oder 2'-Desoxyguanosin-5'-monophosphat ist.

# Revendications

1. Un procédé de production de 3'-désoxyguanosine selon lequel on fait réagir un dérivé de guanine de formule I ci-dessous:



[I]

(dans laquelle R désigne un atome d'hydrogène ou un groupe ribose-1-yle ou 2-désoxyribose-1-yle ou un monophosphate, diphosphate ou triphosphate de celui-ci) avec un donneur de 3-désoxyribose en présence d'une source de nucléoside phosphorylase pour former la 3'-désoxyguanosine.

2. Un procédé selon la revendication 1 dans lequel le donneur de 3-désoxyribose est choisi parmi la 3'-désoxyadénosine, la 3'-désoxy-inosine et leurs mono-, di- et triphosphates et le 3-désoxyribose-1-phosphate.

3. Un procédé selon la revendication 1 ou 2 dans lequel la source de nucléoside phosphorylase provient d'un microorganisme.

4. Un procédé selon la revendication 3 dans lequel source de nucléoside phosphorylase est une culture, une masse de cellules intactes ou des cellules modifiées d'un microorganisme du genre *Pseudomonas*, *Brevibacterium* ou *Achromobacter*, ayant la capacité de produire la 3'-désoxyguanosine à partir du dérivé de guanine de formule I et du donneur de 3-désoxyribose.

5. Un procédé selon la revendication 3 dans lequel source de nucléoside phosphorylase est une culture, une masses de cellules intactes ou des cellules modifiées d'un microorganismes *Micrococcus* ou *Staphylococcus*, ayant la capacité de produire la 3'-désoxyguanosine à partir du dérivé de guanine de formule I et du donneur de 3-désoxyribose.

6. Un procédé selon l'une quelconque des revendications 1 à 5 dans lequel le dérive de guanine est la guanine elle-même, la guanosine, la 2'-désoxyguanosine, le guanosine-5'-monophosphate, le guanosine-5'-diphosphate, le guanosine-5'-triphosphate ou le 2'-désoxyguanosine-5'-monophosphate.